ERRATA - CORRIGE

L'abstract qui di seguito riportato si riferisce ad una comunicazione presentata a SIBioC 2004, 36° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica, Padova, 8-11 Giugno 2004. L'abstract originale, pubblicato sul numero di BC dedicato agli atti del Congresso, conteneva numerosi errori tipografici. Viene qui riproposto in forma tipograficamente corretta.

A SIMPLE ASSAY TO DETERMINE TOTAL HOMOCYSTEINE AND OTHER THIOLS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND FLUORESCENCE DETECTION

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INTRODUCTION: Homocysteine (Hcy), a thiol-containing amino acid, is intimately related to methionine and cysteine metabolism. An elevated concentration of fasting plasma total homocysteine (tHcy) is a risk factor for many diseases ranging from cardiovascular diseases to pregnancy-related diseases. Pregnancy-associated hyperhomocysteinemia is associated with adverse effects of pregnancy such as neural tube defects, abruptio placentae, and preeclampsia. Hyperhomocysteinemia results from enzyme and/or vitamin deficiency. The determination of tHcy levels may provide information for a possible aetiological connection between two apparently unrelated diseases of infancy and adult life: neural tube defects (NTD) and coronary heart disease (CHD).

OBJECTIVE: This abstract describes a modified, rapid, user-friendly, HPLC-FD method for the determination of tHcy and other thiols of interest from only 100μL of plasma.

MATERIAL AND METHODS: SBDF, D,L-homocysteine, L-cysteine, cysteinyl-glycine, GSH, TNBP were purchased from Sigma-Aldrich. We studied tHcy plasma concentrations in 21 healthy subjects (15 men and 16 women; mean age, 43.8±10.6 and 40.7±9.0 years, respectively). Briefly: 100 μL of plasma and 10 μL of the TNBP reagent was incubated for 30 min at 4°C after which 100 μL of TCA was added. The clear supernatant was added to an eppendorf containing 10 μL of 1.55mol/L NaOH; 100μL of 0.125mol/L borate buffer, pH 9.5 and 10 μL of SBDF solution. The sample was then incubated for 20 min at 60°C. The HPLC system consisted of a 600E Waters pump, Waters 474 fluorescence detector (390 nm ex, 515 nm em) and Waters 746 Data Module. Separation of the SBDF derivatized plasma thiols was performed on a Spherisorb ODS2 (45°C), 0.1mol/L acetic acid-acetate bufer, pH 4.0 as mobile phase.

RESULTS: Using the above-described optimized chromatographic conditions, the elution times for these amino acids are: Cys = 9.19±0.16, Cys-gly = 13.84±0.5, Hcy = 15.28±0.23, GSH = 21.8±0.47 minutes (means ±SD). Calibration curves for homocysteine were linear up to 200 mol/L for sample prepared in Milli-QH_20 (r^2 = 0.997). The normal reference ranges for tHyc was 3.1-12.9 μmol/L, for men, and 2.0-8.6 μmol/L, for women. The sensitivity associated to the semplicity and the lower cost of the HPLC method developed in our laboratory render it useful for future research.

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REFERENCE

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